Journal of Medical Virology 61:195–200 (2000)

Lack of Protection Against Vertical Transmission of HIV-1 by Interferons Produced During Pregnancy in a Cohort From East African Republic of Malawi

Vladimir Zachar,[1,2]* Gabriella Fazio-Tirrozzo,[3] Trine Fink,[1] Drucilla J. Roberts,[4] Robin L. Broadhead,[5] Bernard Brabin,[3,6] and Peter Ebbesen[1]

[1]*Department for Virus and Cancer, Danish Cancer Society, Aarhus, Denmark*
[2]*Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovak Republic*
[3]*University of Liverpool, Liverpool School of Tropical Medicine, Liverpool, England*
[4]*Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts*
[5]*Department of Pediatrics, University of Malawi, Queen Elisabeth Hospital, Blantyre, Malawi*
[6]*Department of Pediatrics, Academic Medical Center, Amsterdam, The Netherlands*

Interferons (IFNs) associated with pregnancy were studied for their possible role in inhibition of vertical transmission of the human immunodeficiency virus type 1 (HIV-1). A study group was composed of 43 HIV-1-positive mothers, of whom 15 transmitted the virus to the offspring and 28 did not. The control group included 48 HIV-1-negative mother-infant pairs. The IFN-α was detected only sporadically in the maternal sera from the groups of transmitters (27%), nontransmitters (21%), and controls (19%). The average levels of IFN-α were low, 16.3 ± 2.5 pg/ml, 21.4 ± 9.9 pg/ml, and 21.3 ± 9.4 pg/ml among the transmitters, nontransmitters, and control subjects, respectively. In the cord blood, IFN-α was detected only on two occasions among transmitters, and on a single occasion in the control group. IFN-β was absent from both maternal and cord blood in the study group, and found to be present in one case in the control group simultaneously in the maternal and fetal sera. In the placentas, on the other hand, both type I and II IFNs were expressed universally in the villous trophoblast, and IFN-α and -β in the stromal macrophages as well. In one case among transmitters, no IFNs were detected; nevertheless, no significant difference with respect to nontransmitters could be confirmed. Our data suggest that although the placental IFNs have an antiviral potential, they are not sufficient to suppress transmission of HIV from mother to infant. ***J. Med. Virol. 61:195–200, 2000.***

KEY WORDS: placenta; perinatal infection; innate immunity

INTRODUCTION

Over the past two decades, ample evidence has been obtained to demonstrate that interferons (IFNs) are expressed constitutively during pregnancy at multiple sites, including the placenta, amniotic fluid, fetal membranes, and various fetal organs and blood [Lebon et al., 1982; Bocci et al., 1985; Duc-Goiran et al., 1985; Taguchi et al., 1985; Howatson et al., 1988; Bulmer et al., 1990; Khan et al., 1990; Paulesu et al., 1991; Whaley et al., 1994; Paradowska et al., 1997]. Less frequently, IFNs appear to be expressed in the maternal blood [Chard et al., 1986; Ebbesen et al., 1995]. Both type I (α and β) and type II (γ) IFNs may be present simultaneously, and their synthesis appears to be subject to a substantial interindividual variability, as well as to a temporal modulation. It has been postulated that the primary role of IFNs produced during gestation is in the recognition and maintenance of pregnancy [Chard and Iles, 1992]. In general, however, IFNs are central to a number of physiologically important functions, that include defense against viral, bacterial, and protozoal infections, control of proliferation and differentiation, and modulation of immune responses [Tyring, 1995]. Thus within the context of the broad scope of IFN effects, it seems plausible that pregnancy-associated IFNs may also participate in protection of the fetus against vertically transmitted viruses. Support for such a notion has been provided by experiments with vesicular stomatitis virus (VSV) in vitro [Paradowska et al., 1996] and by a population study of

Grant sponsor: European Community; Grant number: TS3 CT92-0059.

*Correspondence to: V. Zachar, Department of Virus and Cancer, Danish Cancer Society, Gustav Wieds Vej 10, DK-8000 Aarhus C, Denmark. E-mail: vladimir@virus.aau.dk

Accepted 8 November 1999

intrauterine herpes simples virus (HSV) infection [Zdravkovic et al., 1997].

Previously, in a study from Nairobi, Kenya, it was observed that high levels of IFN-α were present concurrently in the maternal and cord blood, and in the placental villous and extravillous trophoblast [Ebbesen et al., 1995]. Further analysis based on the same cohort suggested that if coexpressed in all three compartments (maternal blood, cord blood, and placenta), IFN-α may suppress the transplacental passage of human immunodeficiency virus type one (HIV-1) [Zachar et al., 1997]. In an attempt to obtain additional support for the protective effect of IFNs against HIV-1 infection acquired perinatally, the original study of the Kenyan population was extended to the Blantyre area of the East African republic of Malawi.

METHODS

Patient Population and Collection of Biological Material

Approval by local health authorities was secured before the onset of the study. Healthy pregnant women attending the Montfort Hospital in Nchalo and the Chikwawa district hospital in Malawi were recruited during the course of 1995 and 1996. After informed consent had been obtained, a sample of peripheral blood was withdrawn at the time of delivery and screened for human immunodeficiency virus (HIV)-specific antibodies (ImmunoComb, DoubleCheck HIV 1&2; Orgenics). This testing provided the basis for initial classification according to the infectivity status. Altogether, 115 mothers positive for HIV and 48 mothers without detectable antibodies were enrolled in the study. Immediately after delivery, a sample of the cord blood was obtained by venipuncture of the umbilical cord vein and the plasma was isolated. At the same time, several placental biopsies, covering the whole thickness of placenta, were secured for histological examination. The tissue samples were fixed in a 4% buffered formaldehyde.

At the age of approximately 7 weeks, a follow-up sample of the child's peripheral blood was withdrawn into EDTA. The cells were separated from plasma by centrifugation and kept frozen as a pellet until analyzed by polymerase chain reaction (PCR).

Serology

The maternal sera, which were found HIV-positive in the initial screening by ImmunoComb assay at the site of collection, were, upon receipt in the central laboratory in Aarhus, retested using an immunoblot assay (INNO-LIA HIV Confirmation, Innogenetics), which allowed discrimination between HIV-1 and -2.

IFN-α and -β were quantitated in both maternal and cord plasma. The IFNs levels were established in 48 mothers who were HIV negative and who formed the control group, and in 43 mothers whose HIV-positivity status was confirmed serologically and who formed the study group. The levels of IFN-α were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Cytoscreen Immunoassay Kit, BioSource International). The assay performed at the sensitivity level of 12.5 pg/ml, as determined on the basis of an international reference standard. The levels of IFN-β were determined using an ELISA kit (Toray-Fuji Bionics). This system warranted a lower sensitivity limit of 3.1 IU/ml. All samples were assayed in duplicate.

HIV DNA Detection by PCR

The HIV-1-specific DNA sequences were sought in the infant peripheral blood cells obtained from the follow-up sample at 7 weeks after the birth, in the group of HIV-1-positive mothers. The qualitative detection of HIV-1 was accomplished using a commercially available system based on PCR-mediated amplification and nucleic acid hybridization (Amplicor HIV-1 Test, Roche Diagnostic Systems), according to the manufacturer's instructions. Before amplification, the samples were analyzed to determine the amount of input DNA template by a fluorometric assay (PicoGreen, Molecular Probes) using a universal reader (Victor, Wallac). Thus, it was possible to ensure that the input DNA corresponded to no less than 150 μl of blood. As a further measure of control of the suitability for PCR of the extracted DNA, a reference β-globin gene was amplified in all samples with the aid of commercially available primers (Perkin-Elmer).

Immunohistology

IFN-α, -β, and -γ were detected in the placentas from the study group, which was comprised of 15 mothers who transmitted HIV to the offspring and 28 mothers who did not transmit the virus. Before immunoperoxidase staining, serial sections of the villous parts of the placentas were treated with a Target Retrieval Solution (Dako) to reveal the antigens. A polyclonal rabbit IgG (PeproTech) was used as the primary antibody. The specificity was controlled using a preimmune rabbit IgG. As a secondary reagent, biotinylated swine anti-rabbit $F(ab')_2$ fragment was employed, and the label was provided by streptavidin complexed with biotinylated peroxidase (StreptABComplexes, Dako). Visualization of the bound anti-IFN complexes was accomplished using diaminobenzidine as chromogen. In the final step, the preparations were counterstained with Mayer's hematoxylin. The concentrations of primary and secondary antibodies were optimized using selected tissue blocks with high levels of IFN expression. To avoid nonspecific binding, a background-reducing diluent (Dako) was chosen to dilute the primary antibodies. The presence of IFNs was quantitated on an arbitrary scale from 0 to +3. To provide an additional level of control for specificity, blocking experiments to abrogate high-level positivity by preincubating of primary antibodies with respective IFNs were undertaken.

Histopathology

The placentas from both the study and the control group were examined for the signs of chronic villitis

and intervillositis. To this end, the blocks comprising maternal part of the placentas were dissected from the lumps of formalin-fixed tissue and embedded in paraffin. The sections were stained by hematoxylin-eosin before permanent mounting. The histopathological evaluation was carried out on the basis of established criteria [Bernischke and Kaufmann, 1995] using an arbitrary scale from 0 to +3.

Statistical Methods

All statistical analyses were carried out using the SPSS 8.0.0 statistical package. Independence between groups was investigated using nonparametric tests, either the Wilcoxon-Mann-Whitney rank sum test for two groups or the Kruskal-Wallis test for more than two groups. The trend in the proportion between placental IFN-α and -β and between placental histopathology and placental IFNs was tested using Spearman's ρ.

RESULTS

HIV Infectivity Status

The reactivity in the serological screening test was used as a criterion for preliminary inclusion in the study group. Of the original pool of 115 seropositive subjects, in only 43 (37%) cases, the follow-up samples from the 7th week of life were received in a quality that enabled subsequent PCR analysis. The mothers with follow-up samples were subjected to confirmatory testing by an immunoblot assay to exclude false reactivity, and in all cases the initial seropositivity could be corroborated. All infections were found to be type 1. The patients nonreactive in the screening test were classified as HIV-negative and asssigned to the control group. Altogether, the control group comprised 48 otherwise healthy mothers.

The transmission of HIV to the infant was used as a parameter whereby the study group was next subdivided into the group of transmitters and the group of nontransmitters. As determined by PCR, the transmitters group comprised 15 mother-infant pairs and the nontransmitters group consisted of 28 mother-infant pairs. There was no statistically significant difference in the timing of the follow-up sampling between the group of transmitters (5–10 weeks; mean 6.7) and the group of nontransmitters (5–15 weeks; mean 6.7) (P = 0.39).

Serum IFNs

Raised levels of IFN-α in the maternal blood were detected in only 4 of 15 (27%) cases in the group of transmitters and in 6 of 28 (21%) mothers in the group of nontransmitters (Table I). The average levels (mean ± standard deviation) appeared rather low, 16.3 ± 2.5 pg/ml and 21.4 ± 9.9 pg/ml in the transmitters and nontransmitters groups, respectively. The rate with which elevated IFN-α was found in the cord blood was even lower than that in the maternal blood. In the group of transmitters, high IFN-α levels were noted merely in two cases (13%), whereas in the group of nontransmitters, not a single case was detected. High levels were present concurrently in the maternal and fetal compartments of only one pair (No. 2; 7%), which was of the transmitters group. IFN-β was not detectable, using the specific EIA test, in any of the groups, in either the maternal or the cord blood.

In the control group of 48 HIV-negative mother-infant pairs, high IFN-α (21.3 ± 9.4 pg/ml) was detected in the maternal blood in 9 cases (19%), and in one of these cases (No. 48), IFN was also found concurrently in the cord blood (Table II). Statistical analysis of covariation confirmed that the levels of maternal IFN-α did not differ significantly either between transmitters and nontransmitters or between infected and noninfected mothers (P = 0.86). In contrast with IFN-α, IFN-β was observed in the control group only in a single case (No. 52; 2%), simultaneously in both maternal (3.6 IU/ml) and cord (21.8 IU/ml) blood compartments.

Placental IFNs

IFN-α was found to be expressed universally in the placentas from both the group of transmitters and the group of nontransmitters (Table I). In only one placenta, IFN-α was not detectable, and this case was from the transmitters group. IFN-β appeared to follow the same pattern as IFN-α, however, the correlation between those two proved significant only when all subjects of the study group (n = 43) were taken into account (P = 0.03). On the whole, two placentas, one from each of the groups, stained negative for IFN-β. Regarding IFN-γ, a substantially lower immunoreactivity was detected (<+2) and the number of placentas scoring negative was also highest. In particular, in the transmitter group, three were found (20%) and in the nontransmitter group 8 (29%) placentas, which were devoid of IFN-γ. Interestingly, in a single case (No. 7; 7%) in the transmitters group, none of the IFNs was expressed by the placental tissue. Overall, no association could be revealed between the expression levels of either of the placental IFNs and vertical transmission of HIV (P = 0.53, 0.64, and 0.32 for IFN-α, -β, and -γ, respectively).

On the cellular level, regardless of what type of IFN, the immunostaining was prominent within the villous syncytiotrophoblast and cytotrophoblast (Fig. 1). Regarding IFN-α and -β, reactivity was also found in the stromal macrophages: the Hofbauer cells. Frequently, the staining did not appear homogeneous, but exhibited a patchy pattern with more or less discrete areas devoid of immunoreactivity. No staining was apparent on control slides with preimmune sera.

Furthermore, to evaluate the possibility that the induction of IFNs in the placental trophoblast was linked to a focal inflammatory process, histopathological assessment of the villous tissue was carried out. To this end, no correlation could be determined between chronic villitis and intervillositis, and the expression of placental IFNs (P = 0.76). Similarly, the villitis and intervillositis did not appear to be associated with vertical transmission of HIV (P = 0.45). Moreover, the

TABLE I. Survey of the Placental Histopathology, Placental IFNs, and IFN-α and -β in the Maternal and Cord Blood in 43 HIV-1-Infected Mothers*

Subject	Chronic villitis/ intervillositis[a]	Placenta			IFN-α (pg/ml)		IFN-β (IU/ml)	
		IFN-α	IFN-β	IFN-γ	Maternal	Cord	Maternal	Cord
1	0	2	1	0	—	—	—	—
2	1	2	1	1	14.3	18.4	—	—
3	0	1	1	1	—	—	—	—
4	0	1	2	1	18.3	—	—	—
5	0	1	2	1	—	69.0	—	—
6	1	1	1	0	—	—	—	—
7	0	0	0	0	—	—	—	—
9	0	1	1	1	—	—	—	—
10	0	1	1	1	—	—	—	—
11	0	1	0	1	—	—	—	—
12	0	2	2	2	—	—	—	—
13	0	1	2	1	18.5	—	—	—
14	0	1	2	1	—	—	—	—
15	0	1	2	1	13.9	—	—	—
16	0	2	2	0	—	—	—	—
17	0	1	0	0	—	—	—	—
18	0	2	1	1	—	—	—	—
19	0	1	2	1	—	—	—	—
20	0	2	2	1	—	—	—	—
21	0	1	1	1	—	—	—	—
22	0	2	1	1	—	—	—	—
23	1	1	1	1	—	—	—	—
24	2	1	1	0	—	—	—	—
25	0	1	1	1	12.6	—	—	—
26	0	1	1	0	20.8	—	—	—
27	0	1	2	0	—	—	—	—
28	0	1	1	0	19.0	—	—	—
29	0	1	2	0	—	—	—	—
30	0	2	2	1	38.5	—	—	—
31	0	3	2	1	—	—	—	—
32	0	3	3	0	—	—	—	—
33	0	1	3	1	18.1	—	—	—
34	0	2	2	1	18.9	—	—	—
35	0	1	1	1	—	—	—	—
36	1	1	1	1	—	—	—	—
37	0	1	2	1	—	—	—	—
38	0	1	2	2	—	—	—	—
39	0	1	1	1	—	—	—	—
40	0	1	1	1	—	—	—	—
41	0	1	2	1	—	—	—	—
42	0	1	2	1	—	—	—	—
43	0	2	1	1	—	—	—	—

—, not detected
*Individuals 1–15 transmitted virus perinatally to their offspring; individuals 16–43 did not.
[a]Both the extent of chronic villitis and intervillositis and the intensity of placental IFN immnoreactivity were expressed on an arbitrary scale from 0 to +3.

incidence of histopathological changes in the study group of HIV-positive mothers (14%) corresponded closely to that found in the control group of HIV-negative mothers (13%) ($P = 0.81$).

DISCUSSION

The investigation of the cohort of 91 pregnant women, from the Nchalo and Chikwawa districts in Malawi demonstrated that IFNs were detected in the maternal and cord blood only sporadically and at low levels. In the placentas, on the other hand, IFNs were expressed universally. Previously, in another investigation of an African population from Nairobi, Kenya, a sporadic increase of IFNs levels in the maternal and cord blood was also reported [Ebbesen et al., 1995]. Nevertheless, the incidence of IFN-α in cord blood in the Kenya study (6%) surpassed that found in the present investigation (3%) approximately two-fold. The most intriguing observation made by Ebbesen et al. [1995] was the simultaneous presence of IFN-α in maternal and cord blood, and placenta, in a small proportion of subjects (4%). Focusing again on an African population, from a rather close geographic area, we were not able to confirm such phenomenon. In light of the fact that the original finding still remains an anecdotal observation, the cases when IFNs are expressed concurrently in all three compartments are obviously very infrequent. Thus, it seems most likely that, rather than having a universal biological significance, the noted concurrence of IFNs during pregnancy

TABLE II. Survey of the Placental Histopathology, and IFN-α and -β in the Maternal and Cord Blood in 48 HIV-Seronegative Mother-Infant Pairs from the Control Group

Subject	Chronic villitis/ intervillositis[a]	IFN-α (pg/ml)		IFN-β (IU/ml)	
		Maternal	Cord	Maternal	Cord
44	0	18.1	—	—	—
45	1	—	—	—	—
46	0	43.0	—	—	—
47	0	20.9	—	—	—
48	0	27.1	20.0	—	—
49	0	13.9	—	—	—
50	1	—	—	—	—
51	1	—	—	—	—
52	0	—	—	3.6	21.8
53	1	—	—	—	—
54	0	14.2	—	—	—
55	1	—	—	—	—
56	0	17.3	—	—	—
57	0	23.7	—	—	—
58	0	13.1	—	—	—
59	1	—	—	—	—

—, not detected.
*Shown are only those cases (n = 16) in which chronic villitis or intervillositis were found or where the IFNs were elevated.
[a]The extent of placental inflammatory changes was expressed on an arbitrary scale from 0 to +3.

was attributable to factors associated specifically with the Kenyan group of patients.

It is noteworthy that in the present study, IFN-α, -β, and -γ were expressed regularly in the villous syncytiotrophoblast and cytotrophoblast. This finding appears to be consistent with previous investigations, which also reported a frequent detection of these IFNs [Howatson et al., 1988; Bulmer et al., 1990; Paulesu et al., 1991]. However, when compared with the study by Ebbesen et al. [1995], which revealed placental IFN-α only in those cases where IFN-α was present simultaneously in the cord blood (38%), in the present report, the proportion of immunoreactive placentas was substantially higher. Although the distinct nature of the two cohorts could account for the observed discrepancy, the differences related to employed laboratory techniques, such as antigen revealing or immunostaining procedures, could also play a part.

The role of placental IFNs during pregnancy remains somewhat controversial. It has been established that, at least in ruminants, the trophoblast IFN-τ is indispensable for successful implantation of the conceptus [Roberts et al., 1990]. In humans, the existence of an analogue of animal trophoblast IFNs had been proposed [Whaley et al., 1994], but its functional importance has not been substantiated. Interestingly, it has been demonstrated that in vitro both the human first trimester and term trophoblast cells can be readily induced to produce IFN-β, and probably multiple variants of IFN-α [Aboagye-Mathiesen et al., 1993; Fink, 1997]. Thus despite the fact that the existence of human IFN-τ remains elusive, there has been accumulated convincing evidence that human trophoblast is a rich source of IFNs.

Although a view has generally been accepted that the placental IFNs play a role in the maternal recognition and maintenance of pregnancy through regulation of immune responses at the maternal-fetal interface [Chard and Iles, 1992], the physiological significance of these IFNs is still a matter of speculation. Since the limited permissiveness of the placental tissue for VSV, HSV-1, and encephalomyocarditis virus has been found associated with constitutively expressed IFNs [Paradowska et al., 1996]; also, the trophoblast-derived IFN-α and -β have been shown effective against VSV in tissue culture [Toth et al., 1990], it appears that placental IFNs might be important for

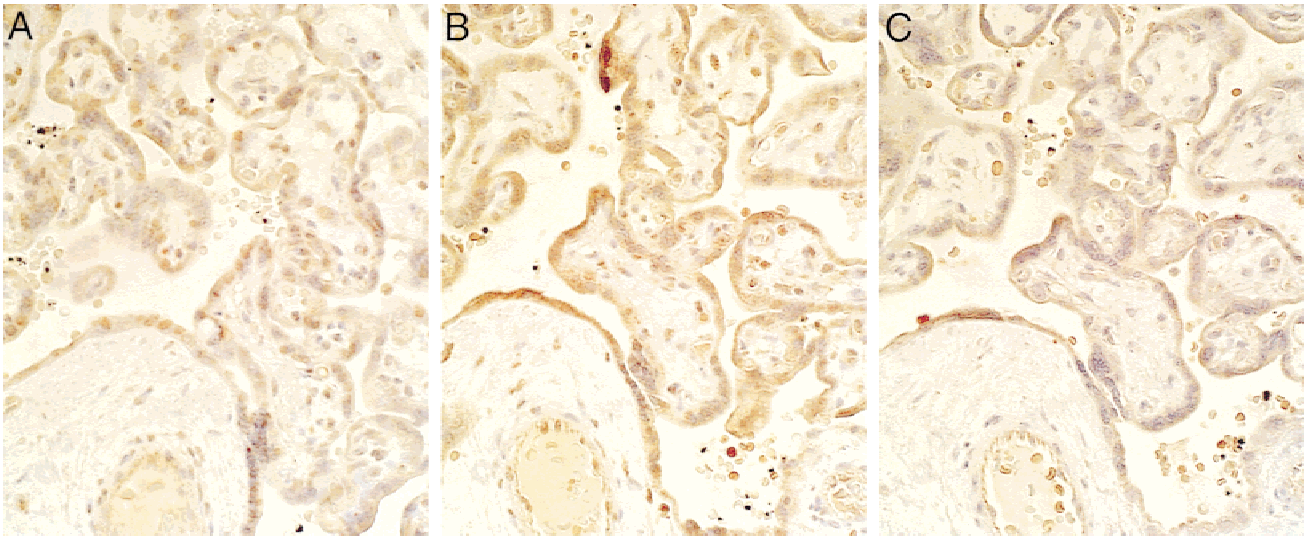

Fig. 1. Expression of interferons (IFNs) in serial sections of a placenta from the cohort of human immunodeficiency virus type 1 (HIV-1)-infected mothers. Immunoreactivity against IFN-α (**A**), -β (*B*), and -γ (**C**) is confined to the villous trophoblast, and with IFN-α and -β also to the stromal macrophages (Hofbauer cells). Staining by indirect immunoperoxidase technique and counterstaining with Mayer's hematoxylin. ×400.

antiviral immunity in a manner similar to that of classical IFNs. Hence, the involvement of IFNs produced by the placenta in the protection of the fetus against vertically transmitted viral infection represents a real possibility, which should be explored further because of its medical significance.

Within this context, it is of interest that HIV is susceptible to treatment with IFNs. HIV has been previously suppressed efficiently by the type I and II IFNs in the lymphoid and monocytic cells in vitro [Coccia et al., 1994], and also by IFN-α in the cultures of trophoblastic cells [Bourinbaiar et al., 1995]. The administration of IFN-α during preclinical stages of HIV infection proved beneficial as well [Skillman et al., 1996; Rivero et al., 1997]. Therefore it is conceivable, that if present in asymptomatic HIV-infected pregnant women in all three compartments in sufficiently high levels, IFNs may bring about some degree of suppression of the virus vertical transmission. Preliminary evidence for such a scenario was furnished when a subgroup of the Kenyan cohort was analyzed [Zachar et al., 1997]. Unfortunately, a limited sample size precluded to draw a more general conclusions. It is interesting to note that, albeit dealing with a different virus, HSV, a recent report by Zdravkovic et al. [1997] also lends support for the role of IFN-α placentally produced for the protection of fetus against intrauterine infection. Nonetheless, in the present study, it was not possible to assess the significance of IFN-α expressed simultaneously in all three compartments for perinatal transmission of HIV owing to merely a single occurrence of such a case. Thus, the role of concurrent and sufficiently high IFN levels in the maternal and fetal circulation, and in placenta in the defense against HIV or other viruses transmitted vertically, still awaits elucidation.

ACKNOWLEDGMENTS

The authors thank Dorte Melsvik, Henry Kamanga, and Zuzana Gulasova for technical assistance. The work was performed at the Danish Cancer Society, Department for Virus and Cancer, Aarhus. This study was supported by grant TS3 CT92-0059 from the European Community (to P.E.).

REFERENCES

Aboagye-Mathiesen G, Toth FD, Petersen PM, Gildberg A, Norskov-Lauritsen N, Zachar V, Ebbesen P. 1993. Differential interferon production in human first and third trimester trophoblast cultures stimulated with viruses. Placenta 14:225–234.

Bernischke K, Kaufmann P. 1995. Pathology of the human placenta. 3rd Ed. New York: Springer-Verlag. 871 p.

Bocci V, Paulesu L, Ricci MG. 1985. The physiological interferon response. IV. Production of interferon by the perfused human placenta at term. Proc Soc Exp Biol Med 180:137–143.

Bourinbaiar AS, Krasinski K, Borkowsky W, Lee-Huang S. 1995. Protective effect of interferon-alpha against cell-mediated human immunodeficiency virus transmission resulting from coculture of infected lymphocytes with fetal trophoblasts. J Interferon Cytokine Res 15:503–508.

Bulmer JN, Morrison L, Johnson PM, Meager A. 1990. Immunohistochemical localization of interferons in human placental tissues in normal, ectopic, and molar pregnancy. Am J Reprod Immunol 22:109–116.

Chard T, Iles R. 1992. Interferon as a fetoplacental signal in pregnancy. In: Miller RK, Thiede HA, editors. Cedard L, Firth A, editors. Placental signals. Autocrine and paracrine control of pregnancy. Trophoblast research. Vol. 6. Rochester, NY: University of Rochester Press. p 55–72.

Chard T, Craig PH, Menabawey M, Lee C. 1986. Alpha interferon in human pregnancy. Br J Obstet Gynaecol 93:1145–1149.

Coccia EM, Krust B, Hovanessian AG. 1994. Specific inhibition of viral protein synthesis in HIV-infected cells in response to interferon treatment. J Biol Chem 269:23087–23094.

Duc-Goiran P, Robert-Galliot B, Lopez J, Chany C. 1985. Unusual apparently constitutive interferons and antagonists in human placental blood. Proc Natl Acad Sci USA 82:5010–5014.

Ebbesen P, Hager H, Norskov-Lauritsen N, Aboagye-Mathiesen G, Zdravkovic M, Villadsen J, Liu X, Petersen PM, Bambra C, Nyongo A, et al. 1995. Concurrence of high levels of interferons alpha and beta in cord and maternal blood and simultaneous presence of interferon in trophoblast in an African population. J Interferon Cytokine Res 15:123–128.

Fink T. 1997. Human trophoblast IFN-alpha variants: expression and biological characterization. In: Thesis. Institute for Molecular and Structural Biology, University of Aarhus. Aarhus: University of Aarhus. 45 p.

Howatson AG, Farquharson M, Meager A, McNicol AM, Foulis AK. 1988. Localization of alpha-interferon in the human feto-placental unit. J Endocr 119:531–534.

Khan NU, Gibson A, Foulis AK. 1990. The distribution of immunoreactive interferon-alpha in formalin-fixed paraffin-embedded normal human foetal and infant tissues. Immunology 71:230–235.

Lebon P, Girard S, Thepot F, Chany C. 1982. The presence of alpha-interferon in human amniotic fluid. J Gen Virol 59:393–396.

Paradowska E, Blach-Olszewska Z, Sender J, Jarosz W. 1996. Antiviral nonspecific immunity of human placenta at term: possible role of endogenous tumor necrosis factors and interferons. J Interferon Cytokine Res 16:941–948.

Paradowska E, Blach-Olszewska Z, Gejdel E. 1997. Constitutive and induced cytokine production by human placenta and amniotic membrane at term. Placenta 18:441–446.

Paulesu L, Bocci V, King A, Loke YM. 1991. Immunocytochemical localization of interferons in human trophoblast populations. J Biol Regul Homeost Agents 5:81–85.

Rivero J, Fraga M, Cancio I, Cuervo J, Lopez-Saura P. 1997. Long-term treatment with recombinant interferon alpha-2b prolongs survival of asymptomatic HIV-infected individuals. Biotherapy 10: 107–113.

Roberts RM, Cross JC, Farin CE, Hansen TR, Klemann SW, Imakawa K. 1990. Interferons at the placental interface. J Reprod Fertil 41(suppl):63–74.

Skillman DR, Malone JL, Decker CF, Wagner KF, Mapou RL, Liao MJ, Testa D, Meltzer MS. 1996. Phase I trial of interferon alfa-n3 in early-stage human immunodeficiency virus type 1 disease: evidence for drug safety, tolerance, and antiviral activity. J Infect Dis 173:1107–1114.

Taguchi F, Kajioka J, Shimada N. 1985. Presence of interferon and antibodies to BK virus in amniotic fluid of normal pregnant women. Acta Virol 29:299–304.

Toth FD, Norskov-Lauritsen N, Juhl CB, Aboagye-Mathiesen G, Ebbesen P. 1990. Interferon production by cultured human trophoblasts and choriocarcinoma cell lines induced by Sendai virus. J Gen Virol 71:3067–3069.

Tyring SK. 1995. Interferons: biochemistry and mechanisms of action. Am J Obstet Gynecol 172:1350–1353.

Whaley AE, Meka CS, Harbison LA, Hunt JS, Imakawa K. 1994. Identification and cellular localization of unique interferon mRNA from human placenta. J Biol Chem 269:10864–10868.

Zachar V, Hager H, Koppelhus U, Zacharova V, Liu X, Bambra C, Goustin AS, Ebbesen P. 1997. Elevated interferon-alpha in maternal and umbilical cord blood and in the placental trophoblast suggests natural protection against vertical transmission of HIV-1 in a Kenyan cohort. AIDS 11:383–385.

Zdravkovic M, Knudsen HJ, Liu X, Hager H, Zachar V, Aboagye-Mathiesen G, Ebbesen P. 1997. High interferon alpha levels in placenta, maternal, and cord blood suggest a protective effect against intrauterine herpes simplex virus infection. J Med Virol 51:210–213.